Physiological and ecological studies on novel carboxydotrophic thermophiles(Digest_要約)

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Summary

In this study, novel hydrogenogenic, thermophilic, carboxydotrophic, and anaerobic bacteria, *Carboxydothermus pertinax* Ug1^T (in Chapter 2) and *Calderihabitans maritimus* KKC1^T (in Chapter 3) were isolated and characterized.

C. pertinax Ug1^T was isolated from an acidic hot spring, Unagi-onsen. Growth of Ug1^T occurred in range of 50-70 °C, pH 4.8-8.6 (optimum at 65 °C, pH6.0-6.5). Strain Ug1^T grew on CO and produced H_2. Strain Ug1^T coupled CO-oxidation with reduction of ferric citrate, amorphous Fe(III)-oxide, AQDS, thiosulfate, and elemental sulfur. Compared to the other members of the genus *Carboxydohermus*, strain Ug1^T was the only strain which can CO-oxidation with sulfur reduction. The 16S rRNA genes of strain Ug1^T were 96.6-94.1% identical to the other members of the genus *Carboxydothermus*. Based on physiological and phylogenetical novelty, strain Ug1^T as a representative of a new species of *Carboxydothermus*, *Carboxydothermus pertinax* sp. nov., is proposed.

C. maritimus KKC1^T was isolated from submerged marine caldera, Kikai Caldera. Growth range of KKC1T were 55-68 °C, pH 5.2-9.2, and 0.8-14% (w/v) salinity (optimum at 65 °C, pH 7.0-7.5, and 2.4% salinity). Strain KKC1^T was able to couple CO-oxidation to reduction of ferric iron, fumarate, sulfite, and thiosulfate. The major products of carboxydotrophy were H_2 and CO_2 while small amount of acetate (<2.0 mM) was also produced. Phylogenetic analysis based on 16S rRNA genes revealed KKC1^T was closest to the members of the genus *Moorella* (<91% similartity). So far, *Moorella* spp. are found in soil, hot springs, and bio-reactors and tolerate up to 2% (w/v) salinity. While none of *Moorella* strain has been isolated from marine environments, KKC1^T can tolerate up to 14% (w/v) salinity. Based on physiological and phylogenetical novelty, strain KKC1^T as a representative of a new species of new genus, *Calderihabitans maritimus*, gen. nov., sp. nov., is proposed.

To discover the unknown ecology of CO-oxidizing microbes in hydrothermal environments, I focused on the members of the genus *Carboxydothermus*, the most studied thermophilic carboxydotrophs found in hot springs, which harbor gene sets of CO metabolisms. A real-time PCR primer set was designed to amplify a conserved region of the gene encoding the CODH-II (*cooS-II*) of *Carboxydothermus* spp. and quantified

their abundance in environmental samples. The highest copy number of *cooS-II* obtained was 9.45 x 10^5 copies/ g sediment from a hot spring, which value was equivalent to 10% of Bacterial 16S rRNA gene copies, while some samples were under detection level (<1.0 x 10^2 copies).

No significant difference of *Carboxydothermus* spp. population was observed between environemtnal and CO enrichment samples; however, *Carboxydthermus* spp. were detectable in various environmental samples, implying that those microorganisms persist in wide range of environments as rare bacteria and ready for growth when environmental conditions allow.

My discovery of physiologically and phylogenetically novel carboxydotrophs from new habitat and detection of *Carboxydothermus* spp. in wide range of environments extends the potential of anaerobic CO-oxidizers that they might prevail in diverse environments and contribute for microbial CO-oxidation.